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## **ENERGY EXPENDITURE, GLUCOSE METABOLISM AND BODY COMPOSITION IN BABOONS AT BASELINE AND AFTER A PARTIAL PANCREATECTOMY AND A 13 WEEKS OF CONTINUOUS INFUSION OF EXENATIDE**

**Tesi di Dottorato di Ricerca**

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## 1. ABSTRACT

Type 2 diabetes mellitus (T2DM) is an extremely complex endocrine and metabolic disease, and the two major causes are impaired insulin secretion and increased insulin resistance in several organs and tissues. A non-human primate is an invaluable model for the study of human diseases due to their close genetic, anatomical and physiological similarities to humans. They are widely used in biomedical research aiming to elucidate the physiological and molecular mechanisms of different diseases, such as cardiovascular diseases, metabolic syndrome, age-related changes in metabolic parameters, obesity, T2DM, atherosclerosis etc.

This entire project has different aims; the first is to confirm that baboons are a very suitable model for studying metabolic diseases such as cardiovascular diseases, metabolic syndrome, age-related changes in metabolic parameters, obesity etc, and confirm baboons as a model in a physical activity studies related to improving health and well-being. In fact, the first study was conducted to evaluate the SenseWear<sup>®</sup> Armband (SWA), a metabolic holter used in humans to estimate the resting and the total energy expenditure in baboon and verify his reliability to estimate energy expenditure during resting and different activities also in non-human primates.

The second and more complex study was related to elucidate, at least in part, the effects of a continuous infusion of a medication for the treatment of T2DM (Exenatide) in a non-human primate, in a model with impaired  $\beta$ -cell function, reporting a insulin sensitizing effect in the Exenatide-treated group, an increase insulin sensitivity and  $\beta$ -cells function and decrease in total body weight. This type of research could be extremely valuable for helping to develop potential new treatments for T2DM.

In conclusion, in the first study we demonstrated that the SWA is a reliable and simple method to estimate total energy expenditure (TEE) and resting energy expenditure (REE) in non-human primate, baboons, by placing it in the “metabolic jacket”.

In the second study we found a powerful, direct, insulin sensitizing effect of EXE on normal glucose tolerant baboons.

These studies provide novel solid basis for further clinical trials aimed at preserving and supporting subjects with diabetes or at high risk of developing it.

## 2. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases of multiple aetiologies characterized by hyperglycemia together with disturbances of carbohydrates, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with microvascular damage affecting, particularly, eyes, kidneys, nerves, and heart, together with an increased risk of macrovascular disease (1).

Type 2 diabetes mellitus (T2DM) is an extremely complex endocrine and metabolic disease, overweight, obesity and physical inactivity are major contributors to the development of insulin resistance and impaired glucose tolerance (2, 3). Recent data published from the World Health Organization (WHO) indicated that:

- ✓ 347 million people worldwide have diabetes.
- ✓ In 2004, an estimated 3.4 million people died from consequences of high fasting blood sugar.
- ✓ More than 80% of diabetes deaths occur in low- and middle-income countries.
- ✓ WHO projects that diabetes will be the 7th leading cause of death in 2030.
- ✓ Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of type 2 diabetes mellitus.

Overt diabetes has diagnosed when one or more of the follow conditions are present:

Criteria for the diagnosis of diabetes (4):

- ✓ Glycated Hemoglobin (HbA<sub>1C</sub>)  $\geq 6.5\%$ .

OR

- ✓ Fasting plasma glucose (FPG)  $\geq 126$  mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.

OR

- ✓ 2-h plasma glucose  $\geq 200$ mg/dL (11.1mmol/L) during an oral glucose tolerance test (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.\*

OR

- ✓ In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose  $\geq 200$  mg/dL (11.1 mmol/L).

The most important condition to avoid developing diabetes is the glucose homeostasis that in T2DM patient is compromised by two major causes: impaired insulin secretion, increased insulin resistance in several organs and tissues.

Pancreatic  $\beta$ -cells are in a constant, dynamic, change state: the balance between islet neogenesis and apoptosis need to be maintained in order to avoid abnormalities in the insulin secretion. Glucotoxicity and lipotoxicity are among the acquired defects that can lead to impaired insulin secretion ([5](#)).

Glucotoxicity of the islet can be defined as a non physiological and potentially irreversible  $\beta$ -cell damage caused by a chronic exposure to a supra-physiological glucose concentration ([6](#)). Reduction in  $\beta$ -cell mass in a condition of basal hyperglycemia can lead to a major impairment in insulin secretion.

Regarding the mechanism by which lipotoxicity can impair  $\beta$ -cell function, the emerging evidence has suggested that long-chain fatty acyl-coenzyme A (Co A) might be involved in the  $\beta$ -cell dysfunction that occurred after a prolonged exposure of free fatty acids (FFAs) inhibiting insulin secretion. Proinflammatory cytokines and leptin, produced by fat tissue, could also impair  $\beta$ -cell function and promote  $\beta$ -cell apoptosis ([5](#), [6](#)).

Insulin resistance has been showed to involve several tissues summarized in the “ominous octet” by Prof. DeFronzo ([7](#)): liver, muscle,  $\beta$ - cell, fat cell, gastrointestinal tract,  $\alpha$ -cell, kidney and brain.

- ✓ Liver: insulin resistance is manifested by an overproduction of glucose at basal state despite hyperinsulinemia, and an impaired suppression of hepatic glucose production in response to food ingestion.
- ✓ Muscle: there is impaired glucose uptake following a meal ingestion leading a postprandial hyperglycemia.
- ✓ As long as  $\beta$ -cell are capable to compensate the insulin resistance increasing insulin secretion glucose tolerance remain normal; whereas, when  $\beta$ -cell start failing plasma glucose start to raise and leads to the onset of diabetes.
- ✓ Lipotoxicity implicates deranges in adipocytes metabolism: fat cells are resistant to the antilipolytic effect of insulin, chronic increase in plasma free FFAs stimulate gluconeogenesis, fail process to secrete normal amounts adiponectin and enlarged fat cell are insulin resistance and have less fat storage capacity.
- ✓ Incretin hormones glucagon-like peptide 1 and glucose-dependent insulinitropic polypeptide (GLP-1 and GIP) stimulate insulin secretion, suppress glucagon secretion, inhibit gastric emptying and reduce appetite; a defect in incretin response to ingestion of a meal lead to a paradoxical raise in plasma glucagon secretion and impaired suppression of hepatic glucose production.
- ✓ Basal plasma glucagon concentration is elevated in T2DM patient that leads to increase basal rate of hepatic glucose production.
- ✓ The ability of the kidneys to reabsorb glucose under hyperglycemia is augmented.
- ✓ Lower posterior and upper posterior hypothalamic areas are key centers for appetite regulation, in T2DM patient the magnitude of the inhibiting response to following glucose ingestion is reduced, leading to increase hepatic insulin production and reducing muscle glucose uptake.

There are several different states occurring between normal glucose tolerance and diagnosis of frank diabetes. Impaired glucose tolerance (IGT) and impaired fasting glycemia (IFG) are



intermediate conditions in the transition between normality to diabetes. People with IGT or IFG are at high risk of progressing to T2DM, although they can remain in those conditions for years before the onset of the disease.

It is possible summarize the evolution of  $\beta$ -cell dysfunction and changes in metabolic parameters during the development of diabetes in 5 stages (8):

1. Compensation: insulin secretion increases to maintain normoglycemia in the face of insulin resistance resulting from obesity, physical inactivity, and genetic predisposition. B-cell mass is tightly maintained through a balance of  $\beta$ -cell birth and  $\beta$ -cell death, there is an increase in  $\beta$ -cell number and probably also  $\beta$ -cell hypertrophy.
2. Stable adaptation: occurs when fasting glucose starts to rise between 5.0-7.3mmol/L.  $\beta$ -cell can no longer be considered to be compensating because truly normal glucose levels cannot be longer maintained, important changes in  $\beta$ -cell function and differentiation occur, acute insulin response (AIR) or first phase is lost.
3. Unstable early decompensation: glucose levels raise relatively rapidly from the range of 7.3 mmol/L to higher values 16-20 mmol/L because of a critical decline on  $\beta$ -cell mass.
4. Stable decomposition: is characterized by a stable decomp -  
50% is lost.
5. Severe decomposition: profound reduction in  $\beta$ -cell mass so severe that people become ketotic and truly dependent on insulin to survive.

Thanks to the progression of the treat of diabetes transition to stage 2 to stage 4 is reversible in T2DM patients with few changes in the lifestyle such as diet control, increasing physical activity and taking drugs.

There are many medical issues that can be investigated and possibly solved with further research in rodents and humans. However, we believe that some of these issues can only be answered through experimental protocols in non-human primates because of their genetic and physiological similarities with humans as our group already showed (9-13).

In both the experimental protocol completed we used non-human primates.

The first study was to evaluate the use of SenseWear<sup>®</sup> Armband (SWA), a metabolic holter used in humans to estimate the resting and the total energy expenditure, in baboon and verify its reliability to estimate energy expenditure during resting and different activities also in non-human primates.

The second and more complex study was related to elucidate, at least in part, the effects of a medication for the treatment of T2DM (Exenatide) in an advanced state of the diabetes disease such as impaired  $\beta$ -cell function. We created a deficiency in insulin secretion by removing part of the pancreas, a procedure called a partial pancreatectomy. With this procedure we intend to prove that the amount of insulin released is decreased after the pancreatic surgery, and that specific pharmacological treatments might preserve insulin production and release, even in the presence of  $\beta$ -cell stress resulting from the surgery. The drug we used is the Exenatide (synthetic exendin-4), a medication approved from the United States Food and Drug Administration (FDA) in 2005 and by the European Medicines Agency (EMA) in 2006 for the treatment of T2DM.

In order to precisely measure the amount of insulin secreted, we created an acute increase in insulin secretory demand by a procedure called hyperglycemic clamp, where we administer a large amount of glucose intravenously in order to maintain a fixed plasma glucose concentration in the bloodstream of the baboons while measuring the corresponding response in insulin production. Before starting the study we expected to observe changes in insulin levels before and after the partial pancreatectomy and also hope to demonstrate that Exenatide improves  $\beta$ -cell function after partial pancreatectomy.

This type of research is extremely valuable for helping to develop potential new treatments for T2DM.

This work has different aims; the first is to confirm that baboons are a very suitable model for studying metabolic diseases such as cardiovascular diseases, metabolic syndrome, age-related changes in metabolic parameters, obesity, T2DM and atherosclerosis etc.

The second is regarding baboons as a model in a physical activity studies related to improving health and well-being.

The third and most important goal is to elucidate the effects of a continuous infusion of Exenatide (synthetic exendin-4) in a non-human primate and the relative effects of the glucose metabolism, and body composition and relative implication for cure T2DM.

### 3. DIABETES

Type 1- and Type 2 Diabetes Mellitus (T1DM, T2DM) are both characterized by a continuous loss of  $\beta$ -cell function paralleled by a progressive decline in  $\beta$ -cell mass (8, 14). At the time of T2DM diagnosis,  $\beta$ -cell function is reduced to  $\sim 50\%$  and  $\beta$ -cell mass to  $\sim 60\%$  (15). In T1DM the deficit in  $\beta$ -cell mass is more severe and averages 70-100% (16). Interestingly, a dimorphic histology of long-lasting T1DM has been reported, with 70% of subjects showing only insulin-deficient islets while 30% numerous insulin-positive cells (17). Indirect evidence of the persistence of residual  $\beta$ -cells 30-40 years after T1DM onset has been recently achieved by using an ultrasensitive C-peptide assay (18).

Different pharmacological interventions have been explored to prevent or reduce the loss of  $\beta$ -cell function and mass in the effort to arrest the progression of these diseases. Unfortunately, as shown by the UKPDS study (19, 20), conventional anti-diabetic drugs do not address this goal and some can actually lead to  $\beta$ -cell death (21). Conversely, more recently developed drugs such as thiazolidinediones (TZD) and glucagon-like peptide 1 (GLP-1) receptor agonists have been shown to induce significant  $\beta$ -cell protection (6). Synthetic long-acting GLP-1 receptor agonists (Exenatide and Liraglutide) exhibit glucoregulatory activities similar to the mammalian incretin GLP-1. These actions include glucose-dependent enhancement of insulin secretion and suppression of glucagon secretion, slowing gastric emptying, reduction of food intake and their clinically effectiveness has been widely demonstrated (3, 22, 23). In addition, GLP-1 receptor agonists show also protective effects on the  $\beta$ -cells, including reduction of apoptosis and enhancement of proliferation and neogenesis (23-27).

However, the vast majority of these studies were performed on rodents and considerably less information is available on human tissues (28, 29). In-vitro, GLP-1 receptor agonists exert anti-apoptotic effects on human  $\beta$ -cells (30, 31) and inhibit the apoptosis induced by inflammation, glucotoxicity, lipotoxicity and reactive oxygen species (32-36). Yet, it remains essentially unknown whether GLP-1 receptor agonists can cause  $\beta$ -cell growth in-vivo in humans or non-human primates.

We and Others have shown that baboons are an excellent animal model for the study of insulin resistance, obesity, and T2DM (9-11, 37). Baboons share 96% genetic similarities with humans.

In baboons physical inactivity and overfeeding induce obesity, insulin resistance ([10](#), [37](#)) and spontaneous diabetes which is phenotypically identical to human disease ([11](#)). Islets of spontaneously diabetic baboons show the same pathological features (i.e. amyloid deposits) of human T2DM pancreases ([9](#)). Because of these remarkable similarities, we further exploited this preclinical model to study the effects of the chronic administration of the GLP-1 receptor agonist exenatide (EXE) on body composition, glucose metabolism, and insulin secretion fate in normal glucose tolerant (NGT) baboons. We could then demonstrate that treatment with EXE induces in this model: - (i) a dramatic increase in insulin sensitivity; - (ii) a compensatory reduction of insulin and C-peptide secretion in response to acute glucose and arginine stimulations. We believe that these findings provide novel worthy rationales for the use of EXE in diabetes prevention and treatment.

## 4. INCRETIN HORMONES

This study aims to look at the effects of glucagon-like peptide-1 (GLP-1) analogue, exendin-4 (Exenatide), on several aspects involved in the development of diabetes, using an animal model. Incretins are hormones secreted by the gut endocrine cells in response to meals ingestion. The most important incretin hormones are glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide (GLP-1). Incretin hormones stimulate insulin secretion, suppress glucagon secretion, inhibit gastric emptying, and reduce appetite and food intake. The “incretin effect” refers to amplification of insulin secretion by hormones secreted from the gastrointestinal tract. Both rapidly stimulate the release of insulin only when glucose level is elevated, thereby enhancing the glucose-sensing and insulin secretory capacity of the endocrine pancreas during postprandial hyperglycemia. (38).

GIP is a 42 amino acid peptide produced predominantly by duodenal and jejunal enteroendocrine K cells in the proximal small intestine; at fasting state the level is low and increases in few minutes after the ingestion of food. GLP-1 is a 30 amino acid incretin hormones produced by enteroendocrine L cells in the distal ileum and colon, and, similar to GIP, its level increases after food ingestion (6). Both GIP and GLP-1 contain alanine in position 2 and are degraded rapidly by dipeptidyl peptidase-4 (DPP-4); furthermore, they are cleared very quickly from the circulation via the kidney.

### 4.1 GPL-1 AND EXENATIDE

GLP-1 acts enhancing glucose-dependent stimulation of insulin secretion, it controls glucose levels by inhibition of glucagon secretion, and it has an inhibiting effect on gastric emptying and reduces the appetite and food intake. Moreover, GLP-1 has a control on the blood glucose level by inhibiting glucagon secretion, suppression hepatic glucose production and decreasing the gastric emptying process; lastly, it influences the central nervous system for the control of satiety (6).

The biological half-life of this hormone is around 2 minutes, and it is degraded by the enzyme DPP-4. GLP-1 mimetics and incretin enhancers (DPP-4 inhibitors) are a new class of drugs used for the treatment of T2DM. (32)

GLP-1 has numerous effects on different parts of the body, acts directly on the endocrine pancreas increasing insulin biosynthesis and beta cell proliferation and reducing apoptosis; on the heart improving myocardial function and cardiac output, on the brain has a neuroprotection effect and reduce the appetite, and on the stomach GLP-1 has a reducing gastric emptying. Whereas GLP-1 acts on liver and muscle indirectly, it may mediate its effects on glucose control independent of insulin secretion through activation of peripheral sensors linked to enhanced glucose disposal in the muscle and inhibiting glucose production in the liver.

All the physiological actions of GLP-1 are summarized in Figure 4.1.1.

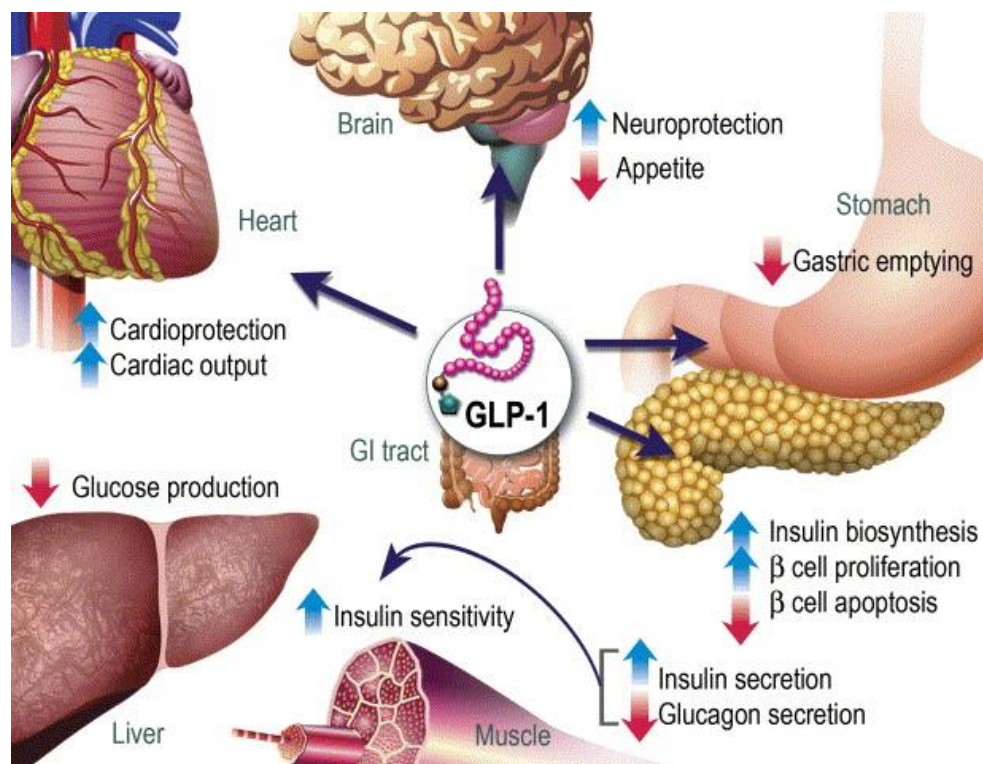


Figure 4.1.1 – GLP-1 actions in peripheral tissues. Figure from Drucker DJ. *The biology of incretin hormones. Cell metabolism.* 2006;3(3):153-65 (39).

Using incretin hormones in treatments for T2DM is very promising; it has been shown to have good results on glycemic profile, insulin sensitivity and  $\beta$ -cell function.

In our studies, we used Exenatide (Amylin Pharmaceuticals, CA) that is a synthetic exendin-4; it is a GLP-1 receptor agonists that have been developed to mimic the insulino-tropic characteristics of endogenous GLP-1 and to resist DPP-4 degradation.([40](#), [41](#)).

Exendin-4 is a 39 amino acid GLP-1 receptor agonist originally isolated from the Gila Monster's (*Heloderma suspectum*) saliva, that shares about 50% sequence of homology to GLP-1, it is a potent agonist for GLP-1 receptor stable against DPP-4 because contains a glycine residue in position 2, thereby conferring resistance to cleavage by DPP-4. Exendin-4 mimics all the glucose-lowering actions of GLP-1. ([39](#)).

Several studies reported the acute effect of GLP-1 and GLP-1 receptor agonist on  $\beta$ -cell that is the potentiation of glucose-dependent insulin release found in animal models and humans ([6](#), [38](#)). The chronic administration of incretins lead to the stimulation  $\beta$ -cell proliferation, induces islet neogenesis associated with increased  $\beta$ -cell mass and inhibition of  $\beta$ -cell apoptosis ([6](#)); moreover, it shows positive effects inhibiting gastric emptying, decreasing body weight and appetite in humans ([3](#), [27](#), [42](#)).

Exenatide has been used in different studies, with positive effects on lowering HbA1c in all treatment groups by about 0.9%, nausea was the principal side effect reported ([43-45](#)); furthermore, it has been shown to decrease concentration of plasma glucose, fructosamine, and free fatty acids as well as it to improve insulin sensitivity and beta cell function in patient with T2DM ([42](#)).

In diabetic patients a continuous administration of GLP-1 lowers blood glucose either at fasting then after food ingestion via inhibition of gastric emptying and glucagon secretion, and stimulation of insulin secretion, decreasing HbA1c, fructosamine, and free fatty acids ([39](#), [42](#), [46](#)). It is also shown that in T2DM patients GLP-1 markedly improves early- and late-phase insulin responses.([42](#)). Moreover, in a recent meta-analyses published by Vilsboll et al., the results indicate that a treatment with GLP-1R agonists helps to reduce body weight in patient overweight and obese, with beneficial effects on systolic and diastolic blood pressure ([3](#)).

Exenatide has effects also on adipocytes, has been shown that induces the expression of adiponectin and simultaneously inhibits proinflammatory adipokines ([47](#)), and it has anti-inflammatory properties on the pancreas islets ([32](#)).



## 5. NON-HUMAN PRIMATES

### 5.1 INTRODUCTION

Non-human primates (NHP) are invaluable models for the study of human diseases due to their close genetic, anatomical and physiological similarities to humans. They are widely used in biomedical research aiming to elucidate the physiological and molecular mechanisms of different diseases, such as cardiovascular diseases, metabolic syndrome, age-related changes in metabolic parameters, obesity, T2DM and atherosclerosis etc. ([48-51](#)).

Common baboons (*Papio sp.*) and macaques (*Macaca sp.*) are the most studied amongst Old World monkeys (*Cercopithecoidea*). In fact, the evolutionary divergence between Hominoidea (humans and apes) and Old World monkeys occurred relatively recently (~ 25 million years ago) and in fact Old World monkeys share great genetic similarities (96 % homology evident at the DNA level) with humans ([10](#), [52](#)).

Baboons (*Papio hamadryas*) are characterized as relaxed and highly adaptable primates that have been largely studied in the wild and have been used in research for over 50 years ([53](#)). They are quadrumanal (pollux and hallux opposable), diurnal and mainly terrestrial with predominant quadrupedal locomotion, have a dense coat of hair, a short or medium-long tail; they are sexually dimorphic (weight and height of these animals is different between species and gender) ([54](#), [55](#)) (Figure 5.1.1).



Figure 5.1.1. Baboon (*Papio hamadryas*) at Southwest Foundation for Biomedical Research. This picture is a kind gift from Dr. Bill Cummins, Associate Director, and Veterinary Resources at the Southwest National Primate Research Center.

Baboons are primarily herbivorous, but they can also be omnivorous, eating small mammals and insects, birds, hares, fish and shellfish. Their average lifespan of ~25 years makes them one of the longest lived primates, which can be maintained in controlled conditions for generations in order to study the effect of genetic and environmental factors ([9](#), [10](#), [53](#)).

Baboons are a valuable research model in different medical fields. They are used as research models for osteoporosis, dyslipidemia, atherosclerosis, nutrition, aging, obesity and insulin resistance ([9](#), [10](#), [13](#), [52](#), [53](#), [56-60](#)). There are many medical issues that can be investigated and possibly solved with further research in rodents and humans. However, we believe that some of these issues can only be answered through experimental protocols in non-human primates because of their genetic and physiological similarities with humans.

Reduction of caloric intake generally improves glucose metabolism and life span through improved insulin sensitivity in rhesus monkeys ([61](#)). Also, increasing the caloric expenditure through exercise can have a positive effect, in the treatment of human obesity and T2DM and other diseases ([62](#), [63](#)).

Regular physical activity has been reported to decrease the risk of a numbers of major chronic diseases ([64](#), [65](#)).

T2DM is one of the major health problems in our society and the incidence of this disease is increasing, currently being the sixth cause of death in the United States ([59](#), [66](#)).

In nature, there is actually no single animal that can replicate the features of T2DM in humans; however, all the actual models (e.g. rodents, cats, dogs, pigs, and nonhuman primates) offer a rich range of opportunities to explore the numerous complexities of T2DM in its facets ([58](#), [66-68](#)).

In obese and old non-human primates the risk of developing diabetes is high similar to humans and they display biochemical features in whole body insulin resistance and a variety of insulin signaling defects in muscle, adipose tissue and liver, during the progression of the disease. Furthermore, pathological changes in the islet of Langerhans, where there was a complete replacement of the islet with an islet-associated amyloid, composed of islet amyloid polypeptide (IAPP), are also speculate to ones seen in patients with T2DM ([9](#), [13](#), [52](#), [69](#), [70](#)).

Another very important non-human primate model of obesity, insulin resistance and T2DM is the rhesus that has been extensively characterized by the group of Barbara Hansen ([71](#)).

## **5.2 NON- HUMAN PRIMATES IN BIOMEDICAL RESEARCH.**

Exercise is recommended to improve fitness, decrease body weight, and reduce the risk of complications of chronic diseases such as obesity, metabolic syndrome and T2DM ([72](#), [73](#)). Therefore, exercise physiology related research is highly relevant to the study of metabolic disease.

Non-human primates have been used in different types of research where physical activity was utilized as a potential intervention to decrease the risk of developing overt diabetes ([59](#)). Previous studies have assessed the effect of exercise in different organs and systems, such as central nervous system (CNS) activity, reproductive organs, nutrition and bone physiology and its effects on body composition. In this regard, in a pilot study Garcia et al ([54](#)) showed that morphometrics and isotope-labeled water can be an appropriate method to study the body composition in baboons. In normal baboons studies they found an average water content of 66% similar to the one previously observed from other studies using different methods, an average fat free mass of about 90% and an average body fat less than 10%. Interestingly, the water content in baboons female is higher than that in woman and reflects the lower body fat in baboons compared to normal women.

At this time there is an increasing interest in studying the contribution of physical activity levels to body weight regulation and body composition. Many of these studies are performed on human subjects, but non-human primate models could help to further understand the molecular mechanisms that occur in humans.

Accelerometers, devices designed to register body movements in any direction have been used to monitor physical activity and consequently estimate energy expenditure ([74](#)). Multiple protocols with accelerometers have been used in humans. These devices have been also used in baboons. In different studies this device has been placed and tested in collars, implanted subcutaneously or placed in a jacket worn by the animals ([64](#), [65](#), [75](#), [76](#)). However, there are others traditional methods well studied in humans such as Double Labeled Water (DLW) ([54](#)), indirect calorimetry (IC) with calculation of Respiratory Quotient (RQ) ([60](#)). These techniques could be potentially applied to baboons and other non-human primates to calculate energy expenditure, but they are

expensive and quite complicated by the fact that they required an active collaboration of the study subject.

New devices called activity monitors are now on the market. Using different mechanisms and software these devices calculate the amount of energy employed in different kinds of activity during the day and they can be worn for several days. These monitors can be useful tools to measure the total energy expenditure during free living activities in humans and non-humans primates.

Non-human primates are particularly useful models for studies in which the physical activity is involved, because of the diurnal patterns of activity similar to the one in humans, but it's not easy to determinate the total amount of energy expenditure that non-humans primates spend leaving in cages with regular tests such as DLW and indirect calorimetry either for the high cost of the treatments that for the high qualifications of the personal involved in the procedures.

To overtake this problem several factories developed devices able to quantify the total movements of the body by 3-ways accelerometers or using multi-sensor activity monitors to have an esteem of energy expenditure in free living activities.

Accelerometers ([64](#), [65](#), [75](#)) are useful to estimate the energy expenditure without interfering significantly in the normal life of the non-human primates. They are used to minimize the external agents, like different cages and different procedure, that can affected the normal behavior of the non-human primate.

Classic techniques have been employed in non-human primates. The indirect calorimetry was performed by a modified metabolic chamber for non-human primates useful to determinate the gas exchange in O<sub>2</sub> and CO<sub>2</sub> during different type of exercises ([53](#), [60](#), [64](#), [77-80](#)) with the additionally calculation of the Respiratory Quotient ratio ([81](#)) as well as the DLW method ([54](#)).

In a recent study Papailiou et al. ([65](#)), used an accelerometer placed in a collar to detect activities that involve the whole body in non-human primates (rhesus monkeys) living in cages. The study demonstrated that the accelerometer is a useful tool for quantifying whole body movements in non-human primates, but it is not possible to detect the amount of energy expenditure for the various behaviors such as chewing and arm movements, only the total energy expenditure was calculated with this device. Additional studies were performed in rhesus monkeys by Sullivan et al. ([82](#)) and Hunnell et al. ([64](#)) and in Marmosets ([75](#)).

In all the studies considered, researchers used different type of exercise and different protocols to evaluate the energy expenditure; studies conducted in non-human primates included three different types of aerobic exercise: running, biking and climbing.

Running on a treadmill and biking on a cycle-ergometer in humans are very common method to evaluate the level of fitness and to training people.

This kind of exercise were also used in non-human primate study; Edgerton et al. (83) in his study trained 11 *Galago senegalensis* for 6 months to run upright rather than on 4 limbs on a treadmill to achieve a 60 min exercise at 43 m/min up to 4<sup>th</sup> grade of inclination. After the training they continue to determinate the differences in muscle properties and structure involved in the exercise training. Also Rhyu (84) in a study on the effect of an aerobic exercise training on cognitive functions, employed 12 *Cynomolgus* monkeys. They were divided in 3 different groups with a different workload and trained 4 of them to run on a treadmill for 1 h a day, 5 days a week for 5 months at the 80% of maximal aerobic power. In addition Ivy et al. (85) evaluated the adaptations by 18 baboons during low to moderate quadrupedal walking exercise on a motorized treadmill.

Another type of exercise used to trained non-humans primates was developed by Hohimer et al. (78, 79) by using a modified chair-ergometer with a special metabolic chamber included. He performed on non-human primates, a dynamic leg exercise using a restrained chair during different exercises observing with dissimilar methods the regional blood flow distribution during the exercise in diverse region on the body.

In additional studies researchers used a different method to achieve the same effect of an aerobic training such as running or biking utilizing the forearm and climbing skill to do that.

Talan et al. (77) in his study trained 3 *Macaca mulatta* to lift weights repeatedly and Bourrin et al. (86) trained 5 rhesus monkeys to execute a rope-climbing exercise for 1 h a day for 5 months, the work was focused to bone mass and bone cellular variations; it showed that this kind of aerobic exercise decrease bone volume and bone formation activity in non-human primates.

Also Zerath (87) trained 5 males *Rhesus macaca* to practice 1h a day climbing in continuous for 5 months to perform an endurance training to demonstrate the changes in bone mass in response to intense aerobic exercise.

Different levels of physical activity are very important in every period of the life to maintain our body in healthy conditions. It's essential to establish the minimal level of activity that people

should perform to be fit, but it's also very important to quantify why in aging for example the physical activity decline, which are the mechanism involved.

Ingram D. (88) and Sallis (89) in different works analyzed the decline in physical activity with age using non-human primates like a model.

Researchers have shown that the age-related decline in activity is observed across a wide range of non-human species and this decline seems to be a predictive factor of lifespan (49, 88, 89). Epidemiological studies have noted the general decline in physical activity with advancing age and that it is generally greater for males than female subjects. It has also been shown in different studies that age is inversely associated with physical activity in studies with children, adolescent and adults. Until now scientists found that the decline in physical activity in humans is greater in males than females especially in the teen years (13-18 year old).

To further understand these arguments scientists have developed different animal models for establishing dose-response relations for various physiological outcomes that could then be further tested and validated in humans.

Physical activity has been demonstrated to produce benefits on human brain functions, influencing brain volume and in the cognitive performances, but the mechanisms involved cannot be easily tested in humans. Rodent models have been established in order to examine the effect of the physical activity on brain structure and functions. Chronic exercise increase the vascular volume fraction in different areas of the cortices and striatum, and increase blood flow in the cerebellum (84).

A similar scientific question was asked in non-human primate, cynomolgus monkeys were trained to run on the treadmill to improve their fitness condition, in order to examine the effect of the exercise on the CNS.

Using non-human primates allowed scientists to standardize and control all of the different factors that can be involved like exercise regimen and lifestyle factors such as diet, stress exposure etc. that cannot be controlled in humans.

Rhyu (84) in his study tested if the regular exercise can help to improve cognitive functions. The exercise regime was the same used in humans, proposed by the American College of Sports Medicine and the American Heart association to improve the level of fitness, in which the subject should run at 80% of the own maximal aerobic capacity. Starting at the 9th week of training, were performed cognitive tests to prove the rate of learning and check how the blood

flow in the brain vary with a regular exercise. This study showed that a regular exercise program at a certain level improved rate of learning and vascular density in the brain in non-human primate.

During exercise there are physiological changes in the blood flow distributions on the different parts of the body.

Baboons, better than other animal model, such as dogs, show interesting similarities in redistribution of cardiac output. Hales et al. (90) tested in awake heat-stressed baboons the difference in blood flow to most of the major organs. Despite small differences he concluded that the splanchnic and renal vasoconstrictions between humans and non-human primates during heat stress is similar in magnitude.

Slightly different were the results in Vatner et al. (91) study, in which non uniform responses in blood redistribution in mesenteric and renal flow during exercise and excitement in non-human primates, were found.

Afterwards Hohimer (79) studied the variation of renal blood flow during a mild dynamic leg exercise in 12 baboons, confirming the previous findings in which baboon shows a decrease renal blood flow during exercise. In a second study (78) he extended the blood redistributions to several organs and tissues and concluded that baboons is a very useful animal model to investigate the different responses of different tissues during exercise

An additional remarkable field in which baboons are very good model is the osteoporosis because they have a bone metabolism and endocrine physiology similar to humans (56, 86, 87).

Aufdemorte et al. (57) analyzed in different pilot studies the differences in bone density between young and aged female baboons using different techniques (radiograph exams, dual X-ray absorptiometry, the histomorphometric analysis, oral bone exams).

They found dramatic differences in bone mass and volume between the two groups, the conclusion was that osteoporosis and oral bone loss is correlated with ovarian dysfunctions. This linkage found in this study was studied also in humans, with similar results.

Also Zerath (87) focused his work to demonstrate the changes in bone mass due to intense aerobic exercise.

Future studies involving genetic factors may help identify genes that can influence the development of the osteopenias in humans (56).



Non-humans primates are a very good model for gynecological physiology ([53](#), [64](#)). Different physiological mechanisms can be affected by incorrect behaviors and can lead to reproductive dysfunctions.

In her study Williams et al. ([53](#)) suggest that a key role in exercise-associated menstrual disorders (EAMD) is the balance between energy intake and energy expenditure. 3 *Macaca fascicularis* were trained to run on a treadmill progressing up to 30 min a day, 7 day a week at 12 km/h to determinate the effect of a very heavy training on the onset of amenorrhea ([53](#)).

Hunnell et al. ([64](#)) checked if the physical activities differ over the menstrual cycle in 7 adults rhesus monkeys recording, using an accelerometer, the activity for several days; results showed that there is no changes across the menstrual cycle in physical activity.

Sullivan et al. in his study ([82](#)) tried to determinate if there is a correlation between the weight gain in adulthood and the level of activity. In this study he tested 18 adult female rhesus monkeys during a 9 months period controlling 4 important parameters such as weight, food intake, level of physical activity and the resting metabolic rate.

During all the period the activity level was measured with a 3-way accelerometer worn on the collar, the food intake was calculated for each meal and the weight was recorded every week. The rest metabolic rate was monitored at the beginning and after 3 months placed the monkey in a metabolic chamber.

Results suggest a very strong correlation between the individual activity level and the tendency to gain weight; higher is the level of physical activity lower is the weight gain.

This study support the data showed also in humans in whom a high level of physical activity prevent or limit weight and fat gain.



### 5.3 SUMMARY

The baboon is a well-established and valuable non-human primate model for the study of multiple human chronic diseases. Currently, the model is subject to intensive research in order to elucidate common underlying mechanisms responsible for human metabolic diseases and the effect of novel pharmacological intervention in the common intricate pathophysiology of obesity, diabetes and the metabolic syndrome. Use and demand of non-human primates has increased in recent decades and several models including baboons have been studied for this purpose with different approaches.

Not surprisingly, accumulating research has shown that metabolic variables both at resting and after exercise, as well as the molecular signaling in adipose tissue and skeletal muscle in baboons highly resemble those in humans, thus providing a natural model for the study of exercise and its effect on adipose tissue, heart, lungs, bone and skeletal muscle metabolism, as well as the fascinating interaction between the “fit” body and the brain.

With the recent identification of newer signaling pathways and molecular targets (i.e. TLR4, NFkB, AMPK) ([63](#), [92-95](#)) that can be modulated by exercise, and the development of novel pharmacological agents designed to enhance the molecular signaling exerted by physical activity, there is an increasing potential for the use of non-human primates as a research model. The inherent morphologic and metabolic characteristics of baboons in combination with improved sensing devices for energy expenditure measurement make them an attractive and valuable model in the future to study the integration between the molecular signaling in skeletal muscle and other metabolically active tissues during physical activity and its correlation with metabolic variables obtained in living conditions.

## 6. ENERGY EXPENDITURE IN NON-HUMAN PRIMATES

### 6.1 INTRODUCTION

The precise evaluation of Energy Expenditure (EE) during free living conditions is important in order to prevent and manage the increasing sedentary lifestyle ([96](#)). In fact, physical inactivity is the fourth leading cause of death worldwide and a critical determinant of many chronic pathological condition such as obesity, insulin resistance and type 2 diabetes mellitus , among others ([97](#), [98](#)).

The gold standards for measuring Resting Energy Expenditure (REE) and Total Energy Expenditure (TEE) in free living conditions are Indirect Calorimetry (IC) and the Double Labeled Water (DLW) techniques, respectively. These techniques are quite elaborate, expensive and can be performed only in few selected centers around the World.

Questionnaire, pedometers and accelerometers were the first alternative methods used to have an accurate and reliable assessment of EE during physical activity ([99-101](#)).

The next generation of these devices is represented by multisensory activity and lifestyle monitors that provide an estimate of REE and TEE and improve the measurement of the physical activity by using different algorithms.

SenseWear® Armband (SWA; BodyMedia, Inc., Pittsburgh, PA) is a multisensory activity monitor. The device is typically worn on the upper right arm and provides estimation of TEE during free living based on a biaxial accelerometer, the galvanic skin response and the body heat loss.

SWA has been validated in different populations including adults ([102-106](#)), children ([107-109](#)) and patients affected by different diseases ([110-114](#)), both during resting ([29](#), [104](#), [106](#), [115](#)) as well of different intensity physical activities ([116-121](#)).

The non-human primate is a valuable animal model in biomedical research, thanks to its close phylogenetic proximity to humans. We have previously demonstrated that baboons display most if not all the critical pathophysiological and molecular alterations that are typically seen in obesity, insulin resistance, and T2DM in humans ([9-11](#), [122-125](#)).

In this study, we assessed in non-human primates, baboons, the REE and TEE by using a new activity monitor called SWA. In humans, SWA is generally placed on the arm, but this body site cannot be used in baboons as they would remove it, in few minutes.

To overcome this problem, we previously demonstrate the reliability of SWA placed the back (latissimus dorsi muscle) in humans and then test the SWA on baboons, in the back, after mounting it in a special jacket that would prevent the animals to remove it.

Therefore, the objectives of this study was evaluate REE and TEE in free living condition in baboons over a period of 6 days with the SWA placed on the latissimus dorsi muscle in a special jacket.

## 6.2 MATERIALS AND METHODS

SenseWear Armband® (SWA) is a wireless multisensory activity monitor; it is normally worn on the upper right arm over the triceps muscle, halfway between the acromion and olecranon. The SWA collects and processes a variety of physiological data through multiple sensors I): a two-axis accelerometer; II): heat flux sensor; III): skin temperature sensor; IV): near-body ambient temperature sensor; V): galvanic skin response sensor that can then be uploaded and analyzed using a computer software called InnerView Research Software (InnerView Research Software 6.1; BodyMedia Inc, Pittsburgh, PA, USA) (Figure 6.2.1).



*Figure 6.2.1. An image of the SenseWear® Armband (SWA).*

### 6.3 NON-HUMAN PRIMATES (BABOONS) SUBJECTS

Twenty one baboons (*Papio hamadryas*), 8 females and 13 males were involved in this study. In order to get anthropometric measurement in the baboons, they were sedated and then weight (kg), height (m) and waist circumference (m) were measured, BMI was calculated as weight (kg)/stature (m<sup>2</sup>); all the anthropometric characteristics are reported in Table 6.3.1.

Primates	Total n=21 mean ± SD	Female n=8 mean ± SD	Male n=13 mean ± SD
AGE (years)	12.2 ± 3.8	15.1 ± 3.2	10.5 ± 2.9
HEIGHT (m)	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
WEIGHT (kg)	27.0 ± 7.4	18.8 ± 0.8	32.1 ± 4.2
WAIST CIRCUMFERENCE (m)	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
BMI (kg/m <sup>2</sup> )	24.8 ± 4.4	21.5 ± 2.4	26.8 ± 4.1

Table 6.3.1. Descriptive characteristics of the baboons (n=21).

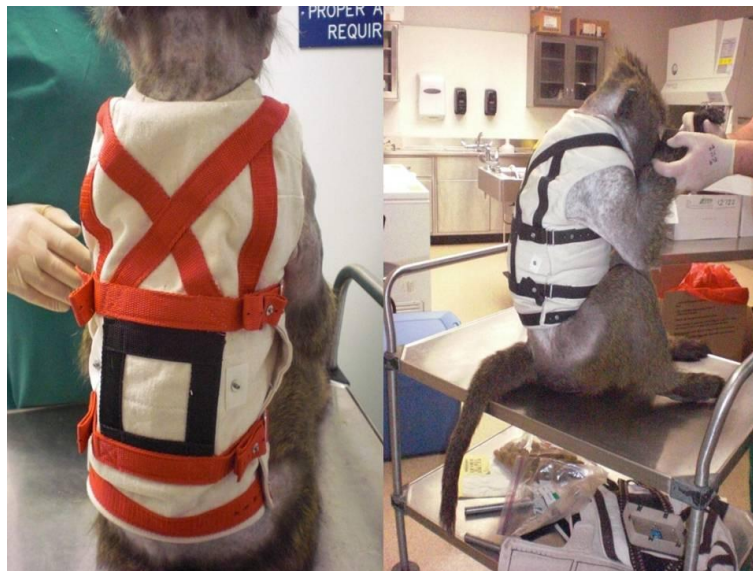
### 6.4 EVALUATION OF FREE LIVING ENERGY EXPENDITURE IN NON-HUMAN PRIMATES

Since it is not possible to keep the SWA on the arm of the baboon because of their innate curiosity and tendency to remove the device, we placed the metabolic holter in a specially designed “metabolic jacket” (Figure 6.4.1) , modified from the one used in the tether system of the baboon that has a slit in the back allowing the placement of the SWA to be in contact with the skin of the baboon’s lumbar area on the latissimus dorsi muscle ([126](#)).



*Figure 6.4.1. Metabolic jacket for energy expenditure measurement in baboons. Device is customized to fit a preset SWA in each baboon's back in order to continuously record energy expenditure over a week (left side). Installation of a metabolic jacket in baboons (right side); in order to ensure continuous reading and data recording a direct and firm contact with the skin is critical.*

We tested the SWA placed in a special jacket in 21 baboons (n= 8 females, n= 13 males) (Figure 6.4.2).



*Figure 6.4.2. Metabolic jackets can be customized and adjusted to different sizes based on baboon gender (Red and white for female; black and with for male), size and age.*

In the first day, the animals were sedated, weight (kg), height (m) and waist circumference (m) were measured and all the needed data inserted in the InnerView Research Software in order to set the SWA before placing it on the jacket.

All animals were housed in a single cage, for 1 week with ad libitum access to water and food (500 g of chow daily plus enrichment such as grains, various kind of fruits and vegetables, peanut butter, dry fruit, honey, cereal, and frozen yogurt). The standard chow contained 57.7% carbohydrates, 15.3% protein, and 4.7% fat (Monkey Diet 15%, Purina 5LE0; TestDiet; Richmond, IN). Enrichment games were constituted by videos and balls.

Baboons were sedated with ketamine to allow placement of the jacket with the embedded activity monitor. Animals were observed twice daily for any clinical or behavioral abnormalities which includes pain or discomfort. Each animal assigned to the study was acclimated to the jacket. Only animals that would accommodate the jackets were selected.

After placement of the metabolic jacket, they were returned to their cage and observed until recovered. The animals were also checked for potential signs of skin irritation produced by the jacket. There were no signs of pain or discomfort produced by the jacket throughout the study period.

After 6 days the animal were sedated again and the jacket with the SWA removed, and the data downloaded.

We evaluated also a REE on 30 min period in which the baboon was not moving and the light was off, and the percentage of contact of SWA with the body surface was 100%.

## **6.5 ETHICS STATEMENTS**

Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Texas Biomedical Research Institute and the University of Texas Health Science Center San Antonio and conformed to the current guidelines of the National Institutes of Health for the care and use of laboratory animals.

## **6.6 STATISTICAL ANALYSIS**

Statistical analysis was performed with GraphPad 5.01 (GraphPad Software, Inc.). The Bland-Altman bias plots were used to assess the agreement between the IC measurement and the SWA estimation (placed in the upper right arm and around the waist in the lumbar zone) during resting and activity. The limit of agreement (LOA) involved the mean difference between the two measurement tools  $\pm 1.96$  SD of the differences.

Pearson's correlations were also used to analyze the correlation between the EE data provided by the SWA, placed on the arm or the back, and IC. Statistical significance was defined at  $p < 0.05$  and data are shown as mean  $\pm$  standard deviation (SD) or mean  $\pm$  standard error (SE).

## **6.7 RESULTS**

In the study we compared the data, provided by SWA placed in the metabolic jacket, during resting and during free living condition in baboons all together and divided by gender.

During the resting part the mean EE was  $0.537 \pm 0.009$  kcal/min for all the animals,  $0.625 \pm 0.005$  kcal/min for males and  $0.367 \pm 0.007$  kcal/min in females.

The total energy expenditure (TEE) has an overall mean of  $0.82 \pm 0.06$  kcal/min,  $0.89 \pm 0.06$  kcal/min for male, and  $0.68 \pm 0.07$  kcal/min for female (Figure 6.7.1).

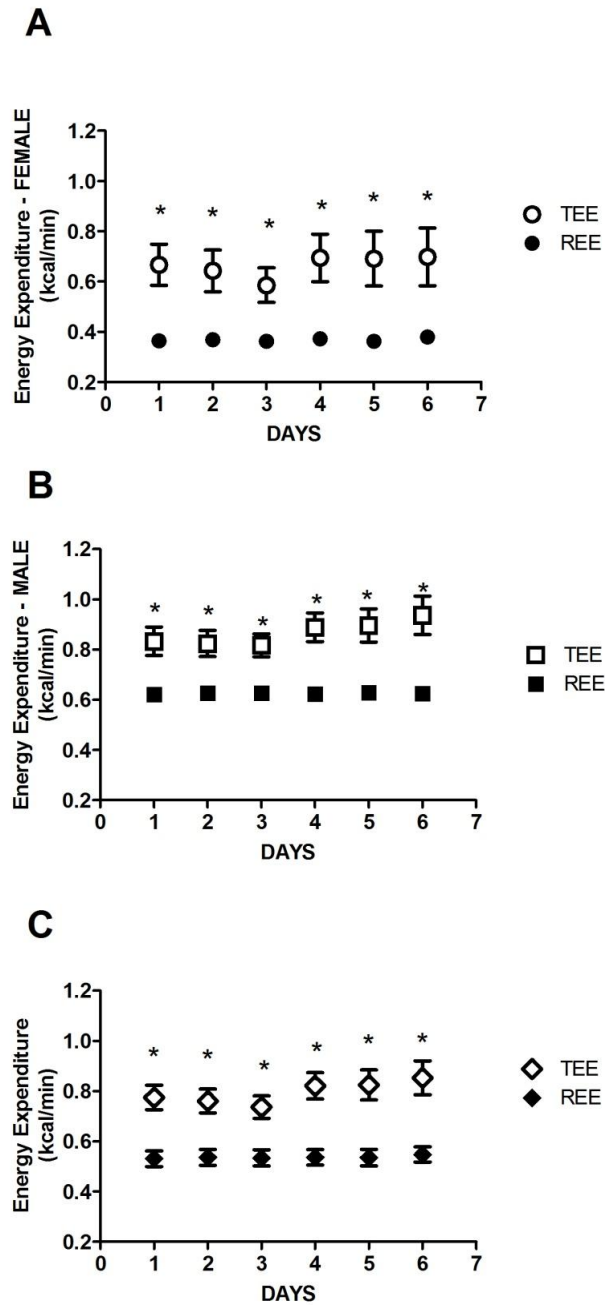


Figure 6.7.1. Baboon Activity (Total Energy Expenditure [TEE] [kcal/day]) and Resting (Resting Energy Expenditure [REE] [kcal/day]). Plots of mean Energy Expenditure (kcal/min) during resting and activity in female (A), male (B) and in all the baboons (C).



## 6.8 DISCUSSION

A large variety of studies have been performed to test the reliability of the SWA by estimating EE during resting and physical activity and comparing the results with DLW ([99](#), [102](#), [103](#), [127](#)), IC ([104-109](#), [111-117](#), [120](#)), accelerometers and pedometers ([74](#)); in controlled situation and in free living conditions ([102](#), [105](#)).

In this study we explored the feasibility to register the EE in baboons placing SWA in a special metabolic jacket for 6 days in free living condition.

In this clinical context, the measurement of the basal metabolic rate can also be important, for example, to provide optimal nutritional support in critical patients.

This study was focused on the adaptation of the SWA in the metabolic jacket for the baboons and measuring energy expenditure during 6 days of free living condition.

We believe that it is interesting to show that this device can be used also in non-humans primate and SWA is able to detect differences in EE during resting and physical activity, also if the EE is very low due to small space (cage) where baboons were placed.

There are very few studies in literature about the daily energy expenditure of baboons.

Rosetta and collaborators measured with DLW, the total EE and compared the variations between two different periods of life, early lactation and after the resumption of sexual cycling, in 8 female baboons (*Papio anubis*); 24 hours total EE was determined over a 4-day period; the average TEE was 3.49 MJ/d in the first period and 3.48 MJ/d in the second ([128](#)).

Leonard and collaborators examined the variations in metabolic requirements among extant primate species based, *Papio anubis*, the Resting Metabolic Rate (RMR) was 956 kcal/d for male and 520 kcal/d for female; the TEE was 1281kcal/d for male and 699 kcal/d for female which are in agreement with our estimated EE employing the SWA ([129](#)). Table 6.8.1 has been included to facilitate the comparison between the present study and the two previously published studies ([128](#), [129](#)).

	TEE (kcal/day)		REE (kcal/day)		Method
	Male	Female	Male	Female	
<b>Present study</b>	1267	936	893	533	SenseWear Armband® (SWA)
<b>Rosetta et Al. (40)</b>	N/A	833	N/A	N/A	Doubly Labeled Water (DLW)
<b>Leonard et Al. (41)</b>	1281	699	956	520	Indirect Calorimetry + Estimation by Equations (Kleiber and Leonard)

*N/A = Data Not Available*

*Table 6.8.1 Comparison of Total Energy Expenditure (TEE) data (kcal/day) and Resting Energy Expenditure Data (REE) data (kcal/day) between three different studies in non-human primates.*

In conclusion, these studies demonstrate that the SWA is a reliable and simple method to estimate TEE and REE in non-human primate, baboons, by placing it in the “metabolic jacket”.

## **7. BABOONS AND EXENATIDE**

### **7.1 RESEARCH DESIGN AND METHODS**

#### **7.1.1 STUDY POPULATION**

Twenty eight female (*Papio hamadryas*) non-diabetic baboons were selected from the colony in the Southwest National Primate Research Center at Texas Biomedical Research Institute (San Antonio, TX) by following the selection criteria reported in a previous study ([10](#)). Each animal was housed in a single cage with ad libitum access to water and food (standard chow containing 57.7% carbohydrates, 15.3% protein, and 4.7% fat- Monkey Diet 15%, 5LE0; TestDiet; Richmond, IN). The amount of food consumed by each animal was registered daily. Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Texas Biomedical Research Institute and the University of Texas Health Science Center San Antonio and conform to the current guidelines of the National Institutes of Health for the care and use of laboratory animals.

#### **7.1.2 STUDY DESIGN**

Prior to treatment baboons underwent a body composition analysis and long-term catheterization for blood collection and drug infusions by placing heparin-coated polyurethane catheters in the internal jugular vein and carotid artery. Catheters were routed subcutaneously to the mid-scapular region where they were connected to the tether jacket chronic infusion system ([126](#)). Three days after catheterization, the 1st 2-step hyperglycemic clamp (HC) was performed followed by a partial pancreatectomy (PPx) removing the tail of the pancreas. After a 96-hrs recovery period on total parenteral nutrition, baboons were fed again with standard chow and randomly divided into 3 study groups: (i) PPx-Exenatide-treated (n=12); (ii) PPx-Saline-treated (n=12), and (iii) Sham-saline-treated (n=4). At the end of treatments, after a 72-hour wash-out period, baboons underwent a 2nd body composition analysis and a 2nd 2-step HC. Baboons were then euthanized and the remnant pancreas removed (Figure 7.1.2.1).

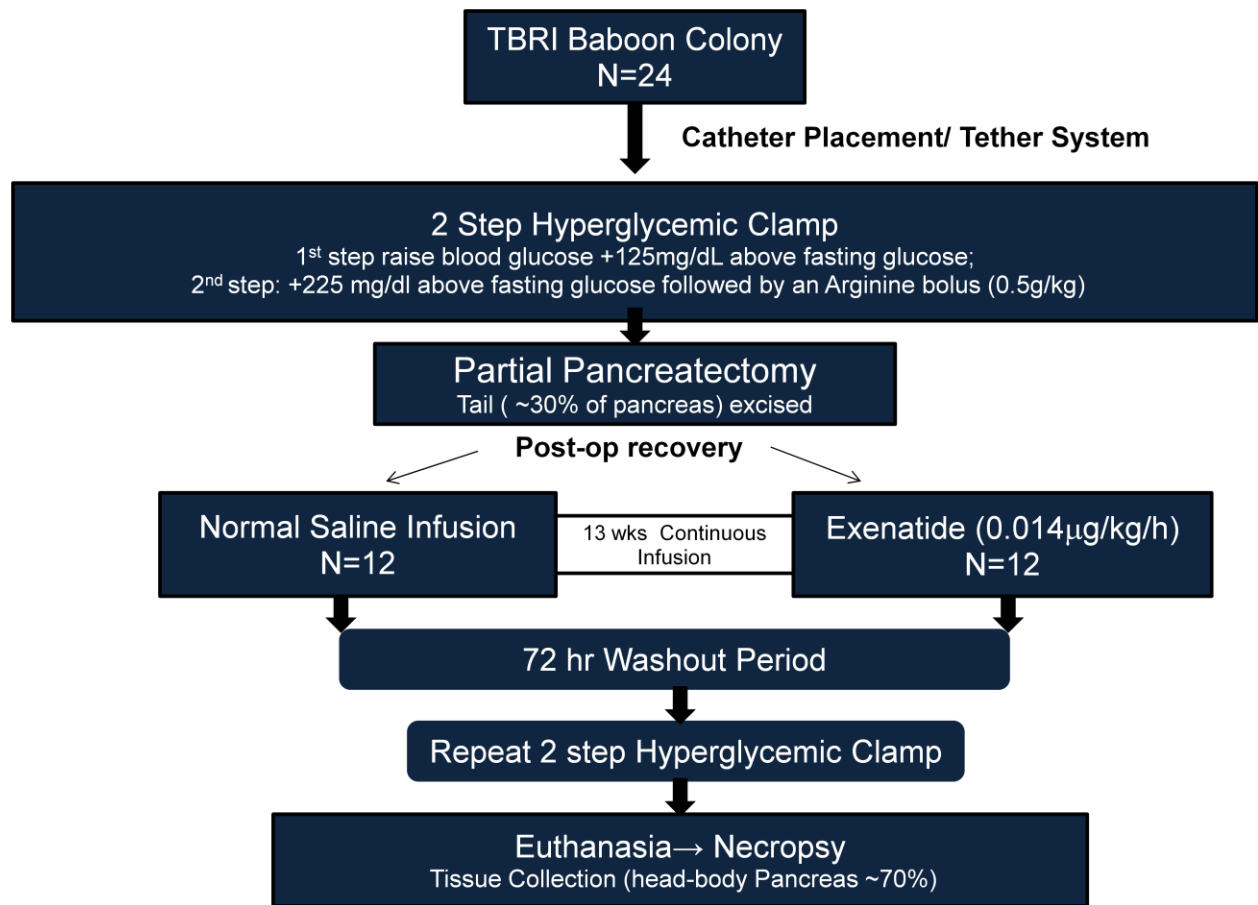


Figure 7.1.2.1. Design of the study.

### 7.1.3 BODY COMPOSITION ANALYSIS

All baboons underwent a dual-energy X-ray absorptiometry (DXA) scan (Lunar Prodigy densitometer; GE Healthcare, Madison, WI) to determine fat-free mass (FFM), fat mass (FM), percent body fat (%BF). Scanning was performed on ketamine-sedated animals and data were obtained by using the software Encore2007 (GE Healthcare, Madison, WI). Animal were placed in a supine position during the scan.

#### **7.1.4 HYPERGLYCEMIC CLAMP**

After an overnight fasting, baboons were sedated with ketamine hydrochloride (10 mg/kg i.m.) and endotracheal intubation was performed using disposable cuffed tubes (6.5-8.0 mm diameter) under direct laryngoscopic visualization. Oxygen was given (98-99.5% FiO<sub>2</sub>) to keep the oxygen saturation > 95% and deep anesthesia induced by using a mix of oxygen and inhaled isoflurane (0.5-1.5%). After animal stabilization, a 2-step HC was performed as originally described ([130](#)). Briefly, a bolus of 50% dextrose (Dextrose 50%, Hospira, Lake forest, IL) solution was administered and followed by a continuous infusion of 20% dextrose (Dextrose 20%, Baxter, Deerfield, IL). The rate of infusion was calculated to raise and maintain plasma glucose 125 mg/dL above basal. After 90 min, the infusion rate was increased to achieve the second hyperglycemic step [(FPG + 125 mg/dl) + 100 mg/dl] which was steadily controlled until the end of the experiment. At time 180 min, an Arginine bolus (10% Arginine Hydrochloride, Hospira, Lake Forest, IL) was administered. Blood samples were collected in EDTA tubes supplemented with 100 µL of Aprotinin. Glucose was measured by using a Glucose Analyzer (GM9 Glucose Analyzer, Analox Instruments USA Inc, Lunenburg, MA) and insulin, glucagon and C-peptide by RIAs.

#### **7.1.5 PARTIAL PANCREATECTOMY (PPx)**

A midline epigastric incision was performed and followed by standard surgical procedures for entering into the abdominal cavity. The free portion of the greater omentum was retracted cranially and the omental leaf bluntly separated to allow direct visualization of the left pancreas. PPx was obtained using the suture fracture technique by making an incision on the mesoduodenum or omentum on each side of the pancreas to mark the portion of it to be removed. Pancreas was then ligated immediately proximal to the marks and the distal part, accounting for ~ 30% of the whole organ, was excised. Holes in the mesoduodenum and omentum were sutured with absorbable material and the abdominal cavity closed. After surgery, baboons underwent a 96-hrs recovery period on total parenteral nutrition (TPN). Baboons of the sham (SHAM) group did not undergo surgery.

### **7.1.6 TREATMENT**

By using the tether system ([126](#)) the baboons were chronically infused intravenously for 13 weeks with either saline (SAL and SHAM groups) or Exenatide (Byetta, Amylin Pharmaceuticals, San Diego, CA) (EXE group) at the dose of 0.014 µg/kg/h. To allow for continuous infusion via the tether system, exenatide was diluted in normal saline.

### **7.1.7 CALCULATION INSULIN SECRETION RATE, INSULIN SENSITIVITY AND DISPOSITION INDEX**

Insulin sensitivity was calculated by M/I index ([130](#)) where M is the glucose infusion rate registered during the hyperglycemic clamp and I is the insulin concentration in the same period analyzed for the M, (150-180 min); is a measure of the quantity of glucose metabolized per unit of plasma insulin concentration .

The disposition index (DI) is the measure of the ability of the β-cell to compensate for insulin resistance; it is calculated multiplying area under the curve of the insulin in the first phase (AUC Insulin (0-12) ) by the insulin sensitivity calculated as a M/I index [AUC Insulin (0-12) \* M/I] ([131](#)).

### **7.2 STATISTICAL ANALYSIS**

Statistical analysis was performed with Stata/SE Version 11.2 (Stata Corp LP, College Station, TX) using non-parametric tests due to a small sample size (n=27). The Wilcoxon rank test was used to make comparisons between groups expressed as percent differences. The Wilcoxon signed-rank test was used for the analysis of the changes from baseline to end of the study. All data were expressed as mean ± SE and statistical significance set for p<0.05. Statistical analysis was performed in collaboration with the Department of Epidemiology and Biostatistics at the University of Texas Health Science Center San Antonio.

## 7.3 RESULTS

### 7.3.1 BODY COMPOSITION ANALYSIS

Total body weight, lean and fat mass were assessed by DXA at the beginning and end of the study. A significant reduction in fat mass was observed in both EXE and SAL-treated baboons (EXE, from  $1.54 \pm 0.45$  to  $0.93 \pm 0.2$ ,  $p=0.04$ ; SAL, from  $1.27 \pm 0.24$  to  $0.86 \pm 0.21$  kg,  $p=0.05$ ) while not in the SHAM group. A slight but significant decrease in total body weight (SAL,  $18.5 \pm 0.7$  to  $16.7 \pm 0.7$  kg,  $p=0.01$ ; EXE  $18.2 \pm 0.8$  to  $17.2 \pm 0.6$  kg,  $p=0.08$ ) and lean mass (SAL  $16.1 \pm 0.5$  to  $15.0 \pm 0.6$  kg,  $p=0.03$ ; EXE  $15.6 \pm 0.4$  to  $15.2 \pm 0.5$  kg,  $p=0.24$ ) was observed only in SAL-treated baboons. The consumption of food during the 13 weeks of treatment in all the three groups was similar and stable (Figure 7.3.1.1).

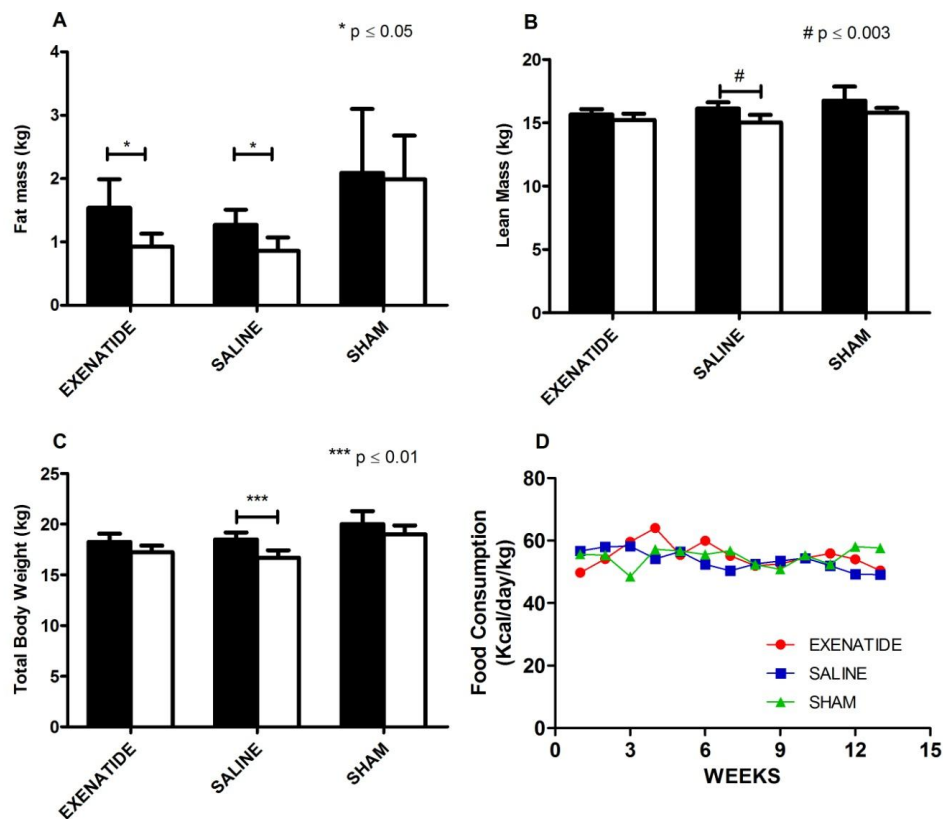


Figure 7.3.1.1. Body composition of all the baboons ( $n=28$ ) at the beginning (black bars) and at the end of the study (white bars), divided by treatment (Exenatide [ $n=12$ ], Saline [ $n=12$ ] and Sham [ $n=4$ ]). A, fat mass (kg), B, lean mass (kg) and C, total body weight (kg). All these data were obtained by DXA. D, average food consumption (Kcal/day/kg of body weight) in the three different groups, during 13 weeks of treatment.

### 7.3.2 SECRETION

All three groups show a slight increase in the area under the curve (AUC) for glucose during the 2nd 2-step HC performed after treatment with EXE or SAL. Differences were not significant in the SAL- (from  $54166 \pm 863$  to  $55969 \pm 1234$ ,  $p=0.21$ ) and SHAM-groups (from  $55901 \pm 790$  to  $56814 \pm 1488$ ,  $p=0.72$ ); however, there was a statistically significant difference in EXE-treated baboons (from  $53697 \pm 711$  to  $57792 \pm 1360$ ,  $p=0.05$ ).

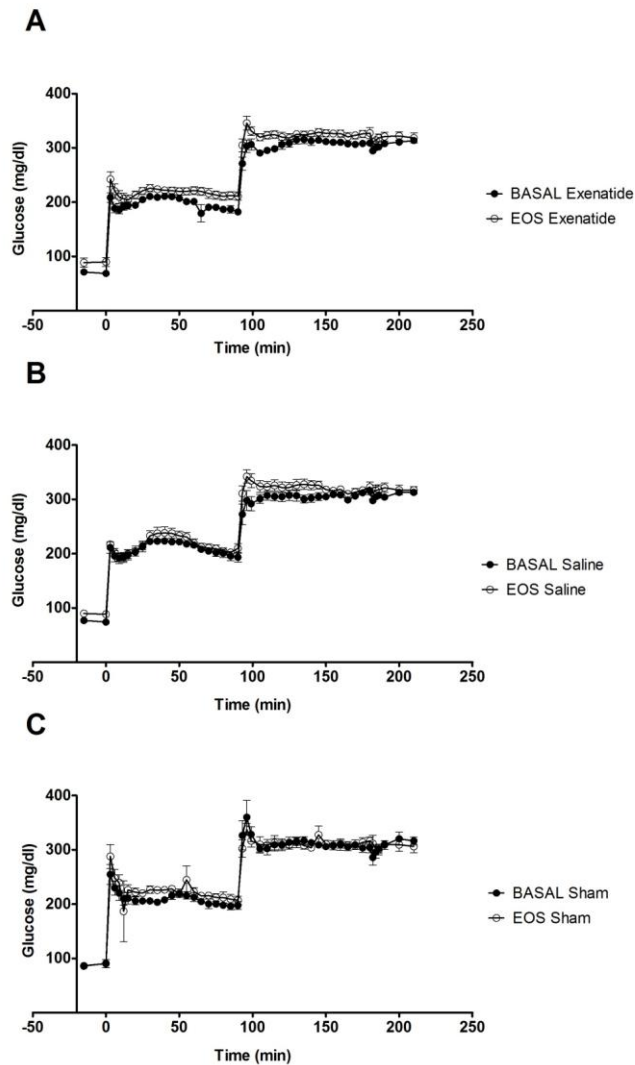


Figure 7.3.2.1. Glucose secretion in the three different groups: Exenatide (A), Saline (B) and Sham (C).



A marked decrease in the AUC for insulin secretion was observed in the EXE group (from  $29243 \pm 5842$  to  $16910 \pm 3432$ ,  $p=0.02$ ), in contrast, insulin secretion remained stable in SAL (from  $16399 \pm 2319$  to  $16862 \pm 3537$ ,  $p=1$ ) and SHAM (from  $29713 \pm 2957$  to  $37993 \pm 12849$ ,  $p=0.47$ ).

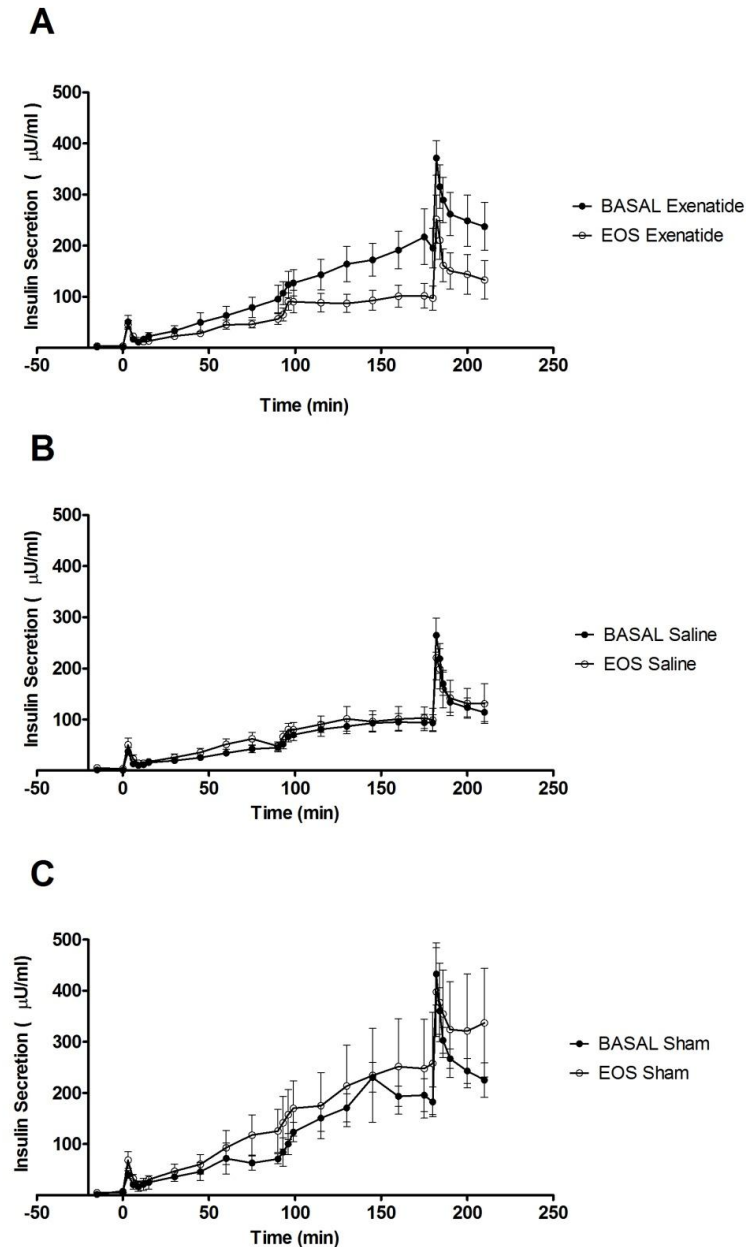


Figure 7.3.2.2. . Insulin secretion in the three different groups: Exenatide (A), Saline (B) and Sham (C).

As expected, AUC for C-peptide secretion showed the same pattern reported for insulin with a significant decrease only in the EXE group (from  $2139 \pm 225$  to  $1654 \pm 221$ ,  $p=0.01$ ) while in SAL (from  $1666 \pm 159$  to  $1574 \pm 141$ ,  $p=0.53$ ) and SHAM (from  $2296 \pm 147$  to  $2675 \pm 425$ ,  $p=0.47$ ) remained stable. In EXE-treated baboons, AUC for insulin and C-peptide secretion was also significantly decreased in response to acute L-arginine stimulation (180-210 min), while not in the other groups.

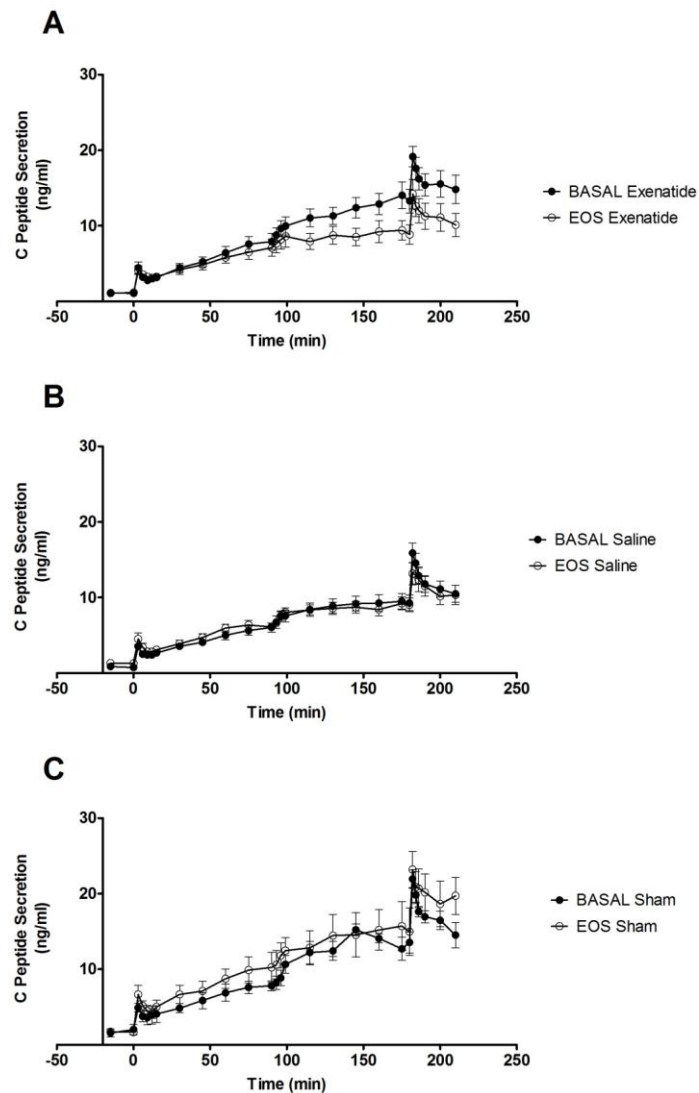


Figure 7.3.2.3. . C peptide secretion in the three different groups: Exenatide (A), Saline (B) and Sham (C).

Finally, a small non-significant increase in the AUC for glucagon secretion was observed in all groups (EXE, from  $5946 \pm 602$  to  $6446 \pm 576$ ,  $p=0.58$ ; SAL, from  $5871 \pm 904$  to  $6362 \pm 876$ ,  $p=0.66$ ; SHAM, from  $8040 \pm 73$  to  $7724 \pm 869$ ,  $p=0.72$ ) (Figure 7.3.2.4). All the data are represented in Figure 7.3.2.5.

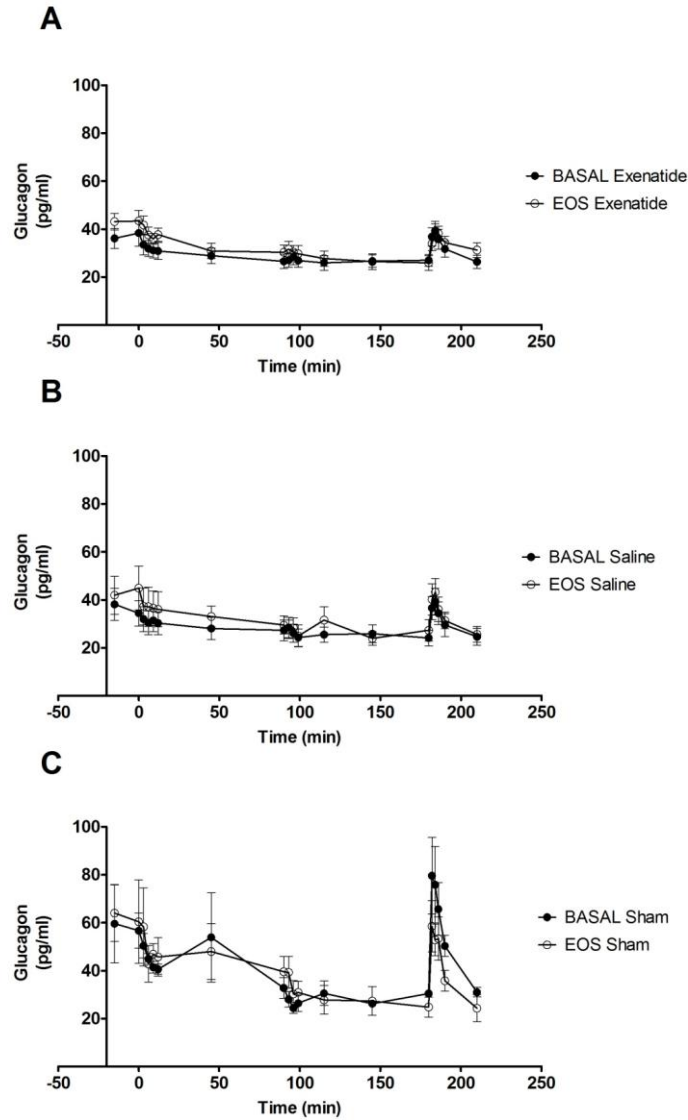


Figure 7.3.2.4. . Glucagon secretion in the three different groups: Exenatide (A), Saline (B) and Sham (C).

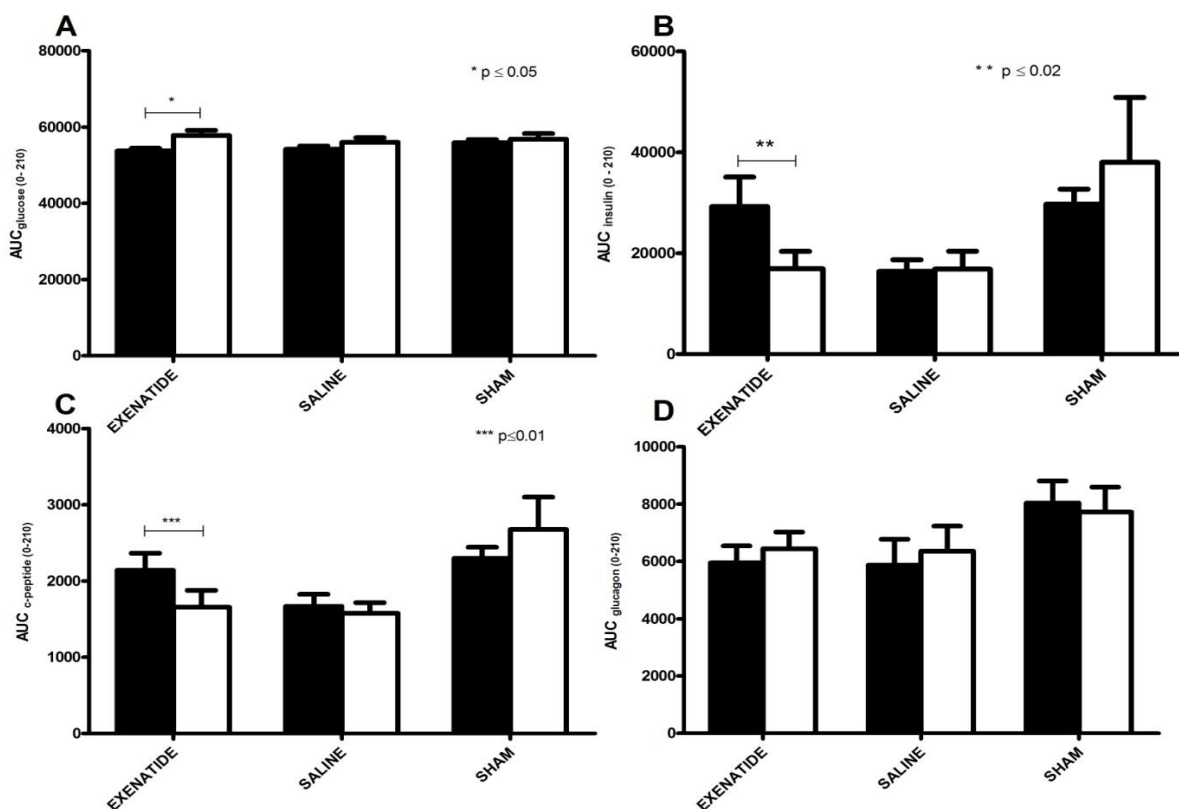


Figure 7.3.2.5 Area under de curve (AUC) evaluation in all the three groups: Exenatide, Saline and Sham, for glucose (A), insulin (B), c-peptide (C) and glucagon (D) secretion.

In EXE-treated baboons was showed a remarkable increase in tissue insulin sensitivity calculated by the M/I index (from  $17.1 \pm 3.1$  to  $28.9 \pm 4.2$ ,  $p=0.01$ ) (Figure 7.3.2.6 B). Conversely, insulin sensitivity did not change significantly in SAL (from  $33.2 \pm 9.2$  to  $26.8 \pm 4.4$ ,  $p=0.86$ ) and SHAM (from  $11.1 \pm 1.6$  to  $10.2 \pm 2.3$ ,  $p=0.72$ ).

Calculation of the  $\beta$ -cell function by using the Disposition Index (DI) showed a significant increase in the EXE (from  $84 \pm 8$  to  $169 \pm 16$ ,  $p=0.01$ ), a small non-significant increase in SAL (from  $125 \pm 19$  to  $151 \pm 20$ ,  $p=0.37$ ) and an insignificant decrease in SHAM ( $79 \pm 29$  to  $63 \pm 19$ ,  $p=0.14$ ) (Figure 7.3.2.6 C).

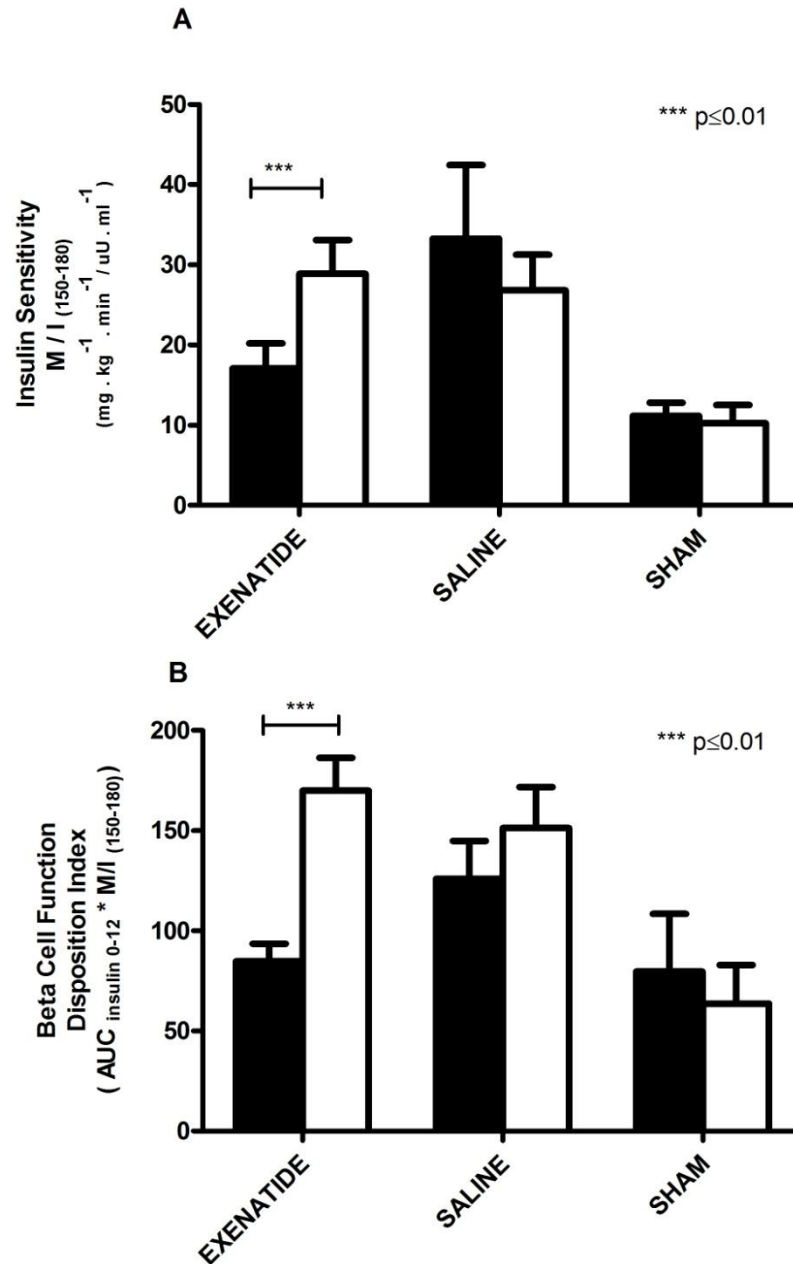


Figure 7.3.2.6. Insulin sensitivity (M/I) [A] and disposition index (DI) [B] calculated by AUC of insulin between 0 to 12 minutes multiply by M/I. All the baboons (n=28) are divided by treatment (Exenatide, Saline and Sham); black bars represent data at the beginning of the study and white bars represent data at the end of the study.

In conclusion, baboons treated with EXE showed a dramatic increase in insulin sensitivity reflecting an improvement in the global  $\beta$ -cell function (or performance) as compared to SAL-treated and SHAM.

## 7.4 DISCUSSION

The major unpredicted finding of this study is the demonstration of the powerful, direct, insulin sensitizing effect of EXE on normal glucose tolerant baboons after partial pancreatectomy. The enhancement of insulin action is not considered amongst the main mechanisms of action of GLP-1 receptor agonists although early pioneering studies have shown that GLP-1 increases significantly insulin sensitivity in both T2DM and T1DM subjects ([132](#)). Thus far, the increased insulin sensitivity commonly observed in subjects treated with GLP-1 receptor agonists, has been considered secondary to the decrease in circulating glucagon levels and body weight ([133](#)). Yet, indication that EXE can improve insulin sensitivity independently from changes in body weight has been already provided in both rodents and humans ([42](#), [134](#), [135](#)). In the present study, treatment with EXE and SAL induced similar effects on body fat mass and circulating glucagon levels suggesting that the increased insulin sensitivity observed with EXE must be due to a direct insulin potentiating effect of this drug. Indeed, acute EXE administration in dogs, sensitizes insulin-mediated whole body glucose disposal and promotes the uptake of exogenous glucose by the liver ([136](#)). The mechanisms mediating the insulin mimetic activity of EXE are currently under investigation.

At the start of the study, baboons underwent a partial pancreatectomy (PPx) that consisted in the removal of part of the pancreatic tail that accounted for by ~ 30% of the whole organ mass. Previous studies in primates and humans have shown that more than 50% of the pancreas mass must be excised to induce hyperglycemia or to increase the risk of developing diabetes ([137-139](#)). As we were not interested with studying the effects of an acute increase in the functional demand on the remnant pancreas, we merely harvested a mass of tissue large enough to allow reliable histopathological comparisons of islets morphology before and after treatments. Surprisingly, however, in spite of the marginal mass of tissue removed, PPx induced a small increase in fasting glucose levels that reached statistical significance in EXE-treated baboons but that was also evident in the control group. Hyperglycemic clamps also showed a modest increase in glucose (AUC glucose). In EXE-treated baboons, this modest increase in AUC

glucose was associated with a remarkable decrease in insulin and C-peptide secretion in response to both glucose and arginine. To our knowledge this is the first report that shows an inhibitory effect of EXE on insulin secretion. The underlying reason EXE-treated baboons showed a small but significantly higher fasting glucose and increased AUC glucose during the clamps remains to be explained. Perhaps, the striking decrease in insulin secretory demand that EXE induced in these baboons is due to quiescent  $\beta$ -cells. Dormancy is a recently recognized  $\beta$ -cell state, far from simple rest, that follows a vigorous decrease in  $\beta$ -cell functional demand such as can be achieved after pancreas transplantation in normoglycemic recipients ([140](#)). Dormant  $\beta$ -cells constitute a functional reserve but the cellular machinery involved in the exocytosis of the insulin granules might be less reactive than normal accounting for the slight increase in plasma glucose levels in response to acute supraphysiologic stimulations. Whether confirmed in humans, the fact that EXE might induce  $\beta$ -cell rest or dormancy is extremely appealing as resting diminishes  $\beta$ -cell immunogenicity ([141](#)) and renders  $\beta$ -cells less vulnerable to the major cytotoxic insults involved in the pathogenesis of T1DM and T2DM ([32](#), [141-143](#)).

In conclusion, in a preclinical model with striking similarities to the human, EXE treatment induces a remarkable insulin sensitizing effect. This study provides novel solid foundation for further clinical trials aimed at preserving the  $\beta$ -cell mass in subjects with diabetes or at high risk of developing it.

## 7.5 TABLES AND FIGURES

Anthropometric Characteristics	EXENATIDE						SALINE						SHAM					
	BASAL			EOS			BASAL			EOS			BASAL			EOS		
	Mean	± SD		Mean	± SD	P-value	Mean	± SD		Mean	± SD	P-value	Mean	± SD		Mean	± SD	P-value
(n=28)																		
Weight(kg)	18.2	± 2.8		17.2	± 2.3	NS	18.5	± 2.2		16.7	± 2.4	0.005	20.0	± 2.6		19.0	± 1.8	NS
Height(m)	0.92	± 0.04		0.92	± 0.04	NS	0.92	± 0.04		0.92	± 0.04	NS	0.89	± 0.04		0.89	± 0.04	NS
BMI(kg/m <sup>2</sup> )	21.7	± 3.0		20.5	± 2.4	NS	21.9	± 2.5		19.7	± 3.0	0.002	25.3	± 4.2		24.0	± 2.9	NS
Waist circumference (m)	0.47	± 0.08		0.44	± 0.06	NS	0.47	± 0.06		0.44	± 0.04	NS	0.52	± 0.11		0.53	± 0.07	NS
Trunk fat mass (kg)	1.0	± 1.3		0.6	± 0.5	0.034	0.8	± 0.6		0.5	± 0.6	0.002	1.5	± 1.6		1.4	± 1.1	NS
Trunk lean mass (kg)	8.2	± 0.7		8.2	± 1.1	NS	8.6	± 0.8		8.1	± 0.9	0.007	8.7	± 1.3		8.4	± 0.6	NS
Trunk total mass (kg)	9.5	± 1.8		9.0	± 1.4	NS	9.7	± 1.3		8.8	± 1.3	0.001	10.4	± 1.8		10.1	± 1.2	NS
Total fat(kg)	1.5	± 1.6		0.9	± 0.7	0.043	1.3	± 0.8		0.9	± 0.7	NS	2.1	± 2.0		2.0	± 1.4	NS
Body fat(%)	7.8	± 6.5		5.3	± 3.3	0.043	6.7	± 3.4		4.9	± 3.0	NS	10.2	± 9.1		10.4	± 6.2	NS
Total lean mass (kg)	15.7	± 1.5		15.2	± 1.7	NS	16.1	± 1.6		15.0	± 2.0	0.024	16.7	± 2.2		15.8	± 0.8	NS

Table 7.5.1 Anthropometric characteristics of 28 baboons involved in the study.



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### Paper :

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### Congress abstracts:

- M. Páez, S. Kamath, F. Casiraghi, A. Davalli, G. A. Abrahamian, A. Ricotti, S. La Rosa, A. Comuzzie, A. Marando, G. Finzi, A. Gastaldelli, E. Dick, R. DeFronzo, G. Halff, F. Folli. Chronic exenatide infusion increases insulin sensitivity, decreases insulin secretion and stimulates islet cell proliferation in partially pancreatectomised baboons . EASD Meeting, Berlin 2012.
- F. Casiraghi, A. Davalli, S. Kamath, G. Abrahamian, R. A. Defronzo, A. O.Chavez, G. Halff, F. Folli. Correlazione tra dati antropometrici, sensibilità insulinica, funzione beta cellulare e marker infiammatori in babbuini normoglicemici. Società Italiana di Diabetologia (SID), Torino 2012.
- F. Casiraghi, A. Davalli, A. O. Chavez, G. Abrahamian, A. Gastaldelli, R. Bastarrachea, K. Subhash, A. Ricotti, P. Zuo, A. Comuzzie, G. Halff, R. A. Defronzo, F. Folli. Exenatide aumenta la sensibilità insulinica e la performance beta cellulare in babbuini sottoposti a pancreatectomia parziale. Società Italiana di Diabetologia (SID), Torino 2012.



- F. Folli, F. Casiraghi, A.M. Davalli,, G.A. Abrahamian, A. Gastaldelli, R.A. Bastarrachea, S. Kamath, R. Petz, P. Zuo, A. Ricotti, Ana Paez, P.B. Higgins, A. Comuzzie, G.A. Halff, R.A. DeFronzo, A.O. Chavez; Continuous Exenatide Infusion Markedly Improves Insulin Sensitivity and Disposition Index in Partially Pancreatectomized Baboons. Is Exenatide the long sought-after “Elixir of Life” for the Islet of Langerhans? CTS-IXA Joint Congress, Miami 2011.
- F. Folli, F. Casiraghi, A.M. Davalli, A. Ricotti S. Kamath, A. Paez, Z. Chang, A. Gastaldelli, G. Abrahamian, G. Halff, R.A. DeFronzo, A.O. Chavez. Baboon as a Model for the Study of Insulin Sensitivity, Beta Cell Function and Subclinical Inflammation following Islet Transplantation. CTS-IXA Joint Congress, Miami 2011.
- F. Casiraghi, A.O. Chavez, A.M. Davalli, G.A. Abrahamian, A. Gastaldelli, R.A. Bastarrachea, S. Kamath, R. Petz, P. Zuo, A. Ricotti, P.B. Higgins, A. Comuzzie, G.A. Halff, R.A. DeFronzo, F. Folli; Continuous Exenatide Infusion Markedly Improves Insulin Sensitivity and Disposition Index in Partially Pancreatectomized Baboons. EASD Meeting, Lisbon 2011.
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- F. Casiraghi, A.O. Chavez, A.M. Davalli , G.A. Abrahamian, A. Gastaldelli, R.A. Bastarrachea, S. Kamath, R. Petz, P. Zuo, P.B. Higgins, Z. Chang, A. Comuzzie, G.A. Halff, R.A. DeFronzo, F. Folli; Effect of Continuous Exenatide Infusion on Insulin Action and Secretion in Partially Pancreatectomized Baboons . Diabetes 60(S1): 2011.

Book:

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